LETTERS TO THE EDITORS

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Chlorazol Black *E* as a Vital Dye

THE use of chlorazol black *E* as a stain for general purposes in ordinary microscopical technique was suggested by Prof. H. Graham Cannon in NATURE some years ago[1]. This nearly black substance agrees with many vital dyes in being a sulphonated acid azo-compound; but whereas trypan blue, trypan red, vital new red, diamine fast scarlet, benzo-purpurine 4*B*, the various Niagara blues, new Bordeaux *L*, Congo rubine, afridol blue, Baumwoll-rubin, etc., all possess two azo-linkages, chlorazol black *E* has three. It was thought interesting to try the trisazo compound as a vital dye, and the results suggest that it may be a useful one.

A 1 per cent suspension of chlorazol black *E* (No. 581 in the Colour Index) in distilled water may be sterilized by bringing to near boiling-point, cooled, and injected subcutaneously into mice in doses of 1 c.c. Injections may be made every day or nearly every day for a week, and the animal killed the day after the last injection. The dye will have spread locally under the skin and coloured it in the vicinity of the injections, but there will not be that general staining of the skin of the whole of the body which occurs with trypan blue and other disazo dyes. Some slight discoloration of the muzzle may be noticed. The internal organs will be found almost to have retained their ordinary colours. If various organs are fixed in Zenker's fluid, embedded in paraffin and sectioned, it will be seen, however, that the dye has been circulating in the blood; for the elements of the reticulo-endothelial system will have taken it up strongly. Striking preparations may be made by staining chromatin with safranine and cytoplasm with orange *G*, against which combination the black particles of the dye show up sharply. The Kupffer cells in the liver will be found to have their cytoplasm loaded with the dye, while the liver cells themselves are free from any particle of it. No trace can be found in any of the kidney cells and the urine is of the ordinary colour; so the dye is apparently not excreted and one can therefore load the reticulo-endothelial system to capacity. Sections of the subcutaneous tissue in the vicinity of the injections show that the histiocytes and fibrocytes have taken up the dye in large quantities.

The difference between the trisazo and disazo compounds in relation to vital staining cannot be attributed simply to the relative sizes of the molecules, for the disazo compound trypan blue has a molecular weight (961) that is actually considerably greater than that of the trisazo chlorazol black (782). One must suppose that particle size rather than molecular weight is concerned, in accordance with the recent findings of Gordon and Chambers[2] on the penetration of acid dyes into cells.

Chlorazol black seems to combine the selectivity of such vital dyes as isamine blue with the rapidity of action of the disazo compounds, and may therefore merit trial by other workers. The specimen used was from batch number 244541 of the British Drug Houses, Ltd. Dr. H. M. Carleton has kindly examined my slides and given me the benefit of his comments on them.

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[1] Cannon, H. Graham, NATURE, **139**, 549 (1937).
[2] Gordon, H. K., and Chambers, R., *J. Cell and Comp. Physiol.*, **17**, 97 (1941).

Parathyroid Glands and Lactation in the Rat

REMOVAL of the thyroid gland together with the associated parathyroid tissue was reported by Nelson and Tobin[1] to have no deleterious effect on lactation, as measured by the growth-rates of litters, in the rat. Contrary results were, however, reported by Folley[2] who, moroever, failed to improve lactation in thyroidectomized rats by injections of thyroxine and small doses of parathyroid extract. Nelson[3] has since confirmed the original findings of Nelson and Tobin. A re-examination of the question in this laboratory, partly in collaboration with Miss H. M. Scott Watson, has reaffirmed the almost total failure of thyroidectomized rats to rear their young and has also shown that administration of much higher doses of parathyroid extract than were previously used results in a significant improvement in lactational performance, which however is still below normal. Typical results are shown in the accompanying table.

Group	No. of rats	No. of pups at 6th day of lactation	No. of pups at 21st day of lactation (weaning)	Mean wt. of pups at 21st day of lactation (gm.)
A	5	38	5	17·4
B	4	30	27	25·6

Group *A*: rats thyroidectomized on 6th day of lactation.

Group *B*: rats thyroidectomized on 6th day of lactation and given daily subcutaneous injections of 10 units of Para-thormone (Lilly) from days 6–9 inclusive and 20 units from days 10–20 inclusive in two cases and from days 10–16 inclusive in the two remaining cases.

These results indicate that the suppression of lactation following removal of the thyroid gland and associated parathyroids is partly due to parathyroid deficiency, a result not altogether surprising in view of the large drainage of calcium from the body during lactation. It must therefore be concluded that the integrity of the parathyroid glands is essential for normal lactation. These experiments are continuing and will be fully reported elsewhere.

I am indebted to Dr. S. K. Kon for generous facilities for working with rats and to Messrs. Eli, Lilly and Co., Ltd., for part of the 'Para-thor-mone' used.

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[1] Nelson, W. O., and Tobin, C. E., *Endocrinology*, **21**, 670 (1937
[2] Folley, S. J., *J. Physiol.*, **93**, 401 (1938).
[3] Nelson, W. O., *Amer. J. Physiol.*, **126**, P. 592 (1939).